# Increases in Peroxidase Isoenzyme Activity in Bean Leaves Exposed to Low Doses of Ozone

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ABSTRACT

Polyacrylamide gel electrophoresis of Tempo bean leaf proteins, extracted from plants grown in filtered air, revealed that low doses of ozone (20-25 pphm for 90-180 min) increased the activity of many peroxidase isoenzymes. Symptomless leaves previously exposed to ozone showed a similar increase in activity, but not as much as in leaves exhibiting definite symptoms of ozone injury. Phytopathology 61:1306-1307.

*Additional key words:* air pollution.

Ozone is an extremely reactive, widespread component of photochemical air pollutants causing foliar injury in a multitude of plants (12). While the symptomatology of ozone injury is well-documented (7), little data are available on in vivo isoenzyme changes occurring in plants exposed to ozone (5, 6). In an effort to assess one aspect of biochemical changes in plants exposed to low doses of ozone, bean leaf peroxidase isoenzymes were examined by the method of polyacrylamide gel electrophoresis. Peroxidase isoenzymes were selected because it is generally known that peroxidase activity increases in response to physiological or environmental stress such as disease or injury. Although ozone induced higher peroxidase levels in white beans, the isoenzymes were not examined (3).

Bean plants, *Phaseolus vulgaris* L. 'Tempo', were cultured from seed in charcoal-filtered air. Tempo bean was selected because the leaves are known to be injured by air pollutants and are more sensitive to ozone than pinto bean leaves (8). Supplemental light of 1,500 ft-c for 10 hr and a day-night temperature range of 30 C-19 C were given until the 14th day after seeding. Plants were then exposed to 25 pphm ozone for 90 min in a special fumigation chamber with a light intensity of 1,800 ft-c, and 85% relative humidity at 26 C. In a second set of experiments, plants 19 days old were fumigated with 20 pphm ozone for 180 min to give an effect equivalent to 14-day-old plants. After fumigation, the plants were immediately returned to charcoal-filtered air in the greenhouse. Twenty-four hr after fumigation, the plants were divided into three groups: (i) untreated and symptomless controls; (ii) symptomless but ozone-treated plants; and (iii) plants showing typical ozone-induced, red-brown lesions on the primary leaves with some collapsed leaf tissue.

A leaf extract (2) was prepared by grinding 5 g fresh wt of chilled, deveined primary leaves from each group in buffer, pH 8.0 (13). The homogenate was filtered through two layers of cheesecloth, and the filtrate was centrifuged 1 hr at 30,900 *g* at 2-4 C. The supernatant fluid was collected and diluted 1:1 with cold buffer prior to disc electrophoresis. Total protein in the preparation was estimated by the method of Lowry et al. (9), with bovine serum as a standard. No significant differences in protein concentration were found among the three groups.

Polyacrylamide gel electrophoresis (4, 11) was performed on primary leaf proteins from plants of each group to determine whether there were any significant protein or peroxidase isoenzyme differences. A 100-μliter fraction of the sample was layered directly on the large pore upper gel. A constant current of 1.25 ma/gel was applied for 20 min and raised to 2.5 ma/gel for the remainder of the run. The peroxidase isoenzymes were visualized by Ornstein's method (1, 2) using benzidine in acetate solution, pH 5.1, and 0.3% $H_2O_2$. The gels were scanned within 2 hr after the end of the run with a spectrophotometer equipped with a linear transport attachment (Gilford Instrument Lab., Inc., Oberlin, Ohio) at 500 nm. The relative absorbance was obtained by using the absorbance in the unstained portion of the gel below the major bands as the reference point. There was no significant difference in absorbance among the gels of the three groups in the unstained region.

Duplicate experiments were performed on 14- and 19-day-old plants giving similar results. Inspection of the stained gels from 19-day-old plants (Fig. 1) re-

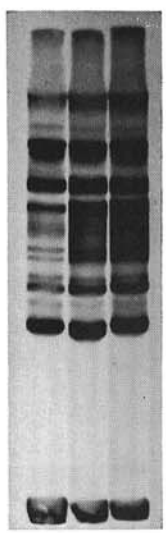

**Fig. 1.** Polyacrylamide gel electrophoresis of peroxidase isoenzymes from bean plants exposed to 20 pphm ozone for 180 min. (Left) Control plants not exposed to ozone. (Center) Plants exposed to ozone but not showing symptoms of ozone injury. (Right) Plants exposed to ozone showing symptoms of ozone injury. The tracking dye moved 5.27 cm from the origin.

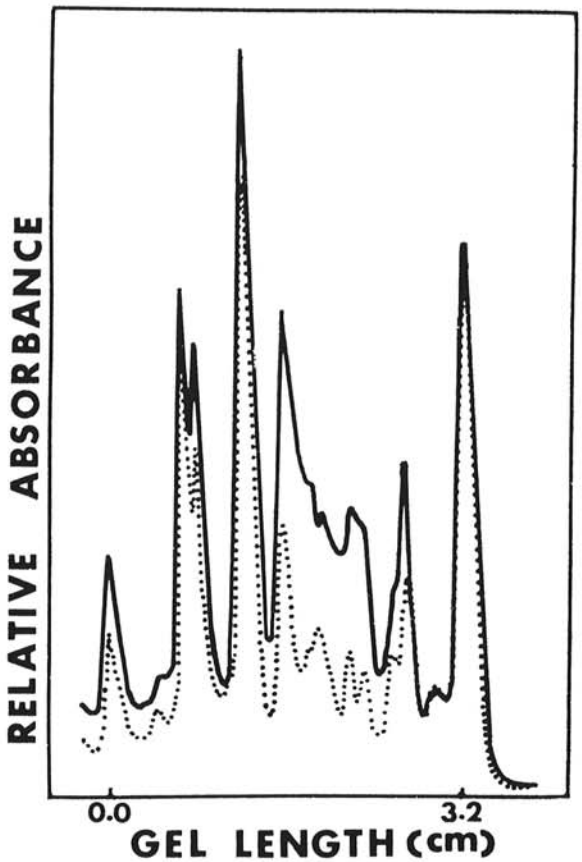


**Fig. 2.** Scan of the polyacrylamide gels shown in Fig. 1 for controls (dotted), and plants showing symptoms of ozone injury (solid). The relative absorbance is shown from the origin to the last major band on the gel. The clear portion of the gel was used as a reference point. Plants exposed to ozone but not having symptoms of ozone injury had a relative absorbance between the controls and exposed plants with symptoms.

veals visual differences in peroxidase isoenzyme activity in the untreated controls (left), symptomless but ozone-treated plants (center), and ozone-treated plants showing symptoms (right). Almost all of the bands showed an increase in intensity as a result of ozone exposure. The leaves with symptoms, however, showed the greatest amount of staining, indicating a greater amount of isoenzyme activity. This was confirmed by examination of the gel scans. Figure 2 shows the scans of the stained portion of the gels relative to the clear area below them. The scans show the relative absorbance of the controls (dotted) and ozone-treated leaves with symptoms (solid). The ozone-treated leaves without symptoms had an intermediate absorbance between these two scans. No new bands were detected as a result of exposure, although there was an over-all increase in background activity which may have obscured any new band with low activity.

The concentrations of ozone used in this study, 20-25 pphm, are somewhat higher than that expected in the ambient atmosphere near Washington, D.C., at peak pollution time. However, ozone is only one injurious component of the photochemical complex to which plants may be exposed. Since ozone and sulfur dioxide are known to act synergistically (10), sensitive plants may be more affected by urban air pollution than is currently believed.

Inasmuch as the exact physiological role of peroxidase in healthy plants is still uncertain, the significance of elevated peroxidase isoenzyme activity in ozone injured plants is unknown. However, our results indicate that polyacrylamide gel electrophoresis could be a useful technique for detecting certain plant biochemical alterations caused by air pollutants even before visual symptoms are apparent.

LITERATURE CITED

1. Canal Industrial Corporation Special Subject Bulletin. Enzyme Anal., Bethesda, Md. 12 p.
2. Curtis, C. R. 1971. Disc electrophoretic comparisons of proteins and peroxidases from Phaseolus vulgaris leaves infected with Agrobacterium tumefaciens. Can. J. Bot. 49:333-337.
3. Dass, H. S., & G. M. Weaver. 1968. Modification of ozone damage to Phaseolus vulgaris by antioxidants, thiols and sulfhydryl reagents. Can. J. Plant Sci. 48: 569-574.
4. Davis, B. J. 1964. Disc electrophoresis II. Methods and application to human serum proteins. Ann. N.Y. Acad. Sci. 121:404-427.
5. Dugger, W. M., & I. P. Ting. 1970. Air pollution oxidants—their effects on metabolic processes in plants. Annu. Rev. Plant Physiol. 21:215-234.
6. Dugger, W. M., & I. P. Ting. 1970. Physiology and biochemical effects of air pollution oxidants on plants. Recent Advances Phytochem. 3:31-57.
7. Heck, W. W. 1968. Factors influencing expression of oxidant damage to plants. Annu. Rev. Phytopathol. 6:165-188.
8. Howell, R. K. 1970. Influence of air pollution on quantities of caffeic acid isolated from leaves of Phaseolus vulgaris. Phytopathology 60:1626-1629.
9. Lowry, O. H., Nira J. Rosebrough, A. L. Farr, & Rose J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.
10. Menser, H. A., & H. E. Heggestad. 1966. Ozone and sulfur dioxide synergism reduced injury thresholds of tobacco leaves. Science 153:424-425.
11. Ornstein, L. 1964. Disc electrophoresis I. Background and theory. Ann. N.Y. Acad. Sci. 121:321-349.
12. Rich, S. 1964. Ozone damage to plants. Annu. Rev. Phytopathol. 2:253-266.
13. Staples, R. C., & M. A. Stahmann. 1964. Changes in proteins and several enzymes in susceptible bean leaves after infection by the bean rust fungus. Phytopathology 54:760-764.